Cellular Immunology 296 (2015) 3–9

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Chicago 2014 – 30 years of γδ T cells

Marc Bonneville [a], Zheng W. Chen [b], Julie Déchanet-Merville [c], Matthias Eberl [d,*], Jean Jacques Fournié [e], Julie M. Jameson [f], Richard D. Lopez [g], Massimo Massaia [h], Bruno Silva-Santos [i]

[a] INSERM UMR 892, Université de Nantes, Nantes, France
[b] Center for Primate Biomedical Research, Department of Microbiology and Immunology in University of Illinois College of Medicine, Chicago, IL, United States
[c] CNRS UMR 5164, Université de Bordeaux, Bordeaux, France
[d] Cardiff Institute of Infection & Immunity, School of Medicine, Cardiff University, Cardiff, United Kingdom
[e] Cancer Research Center of Toulouse, INSERM UMR 1037, Université Paul Sabatier, Toulouse, France
[f] Department of Biological Sciences, California State University San Marcos, CA, United States
[g] Division of Cellular Therapy/Bone Marrow Transplant, Duke University Medical Center, Durham, NC, United States
[h] Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy
[i] Instituto de Medicina Molecular, Universidade de Lisboa, Lisbon, Portugal

ARTICLE INFO

Article history:
Received 18 September 2014
Accepted 1 November 2014
Available online 8 November 2014

Keywords:
γδ T cells
Meeting report
Innate immunity
Adaptive immunity
Infection
Cancer
Autoimmune disease
Inflammation

ABSTRACT

The international γδ T cell conference takes place every 2 years. After being held in Denver (USA) in 2004, La Jolla (USA) in 2006, Marseille (France) in 2008, Kiel (Germany) in 2010 and Freiburg (Germany) in 2012, the γδ T cell community gathered this time in Chicago (USA). This conference was organized by Zheng Chen from 16 to 18 May 2014 at his home institution, the University of Illinois College of Medicine, and boasted 180 attendants from all over the world and almost 100 submitted abstracts.

1. 1984–1987: the discovery of γδ T cells

> "*Whether they are still critical to host defense, or whether they represent an evolutionary relic, is not clear.*" – Immunobiology, ed. Charles A. Janeway and Paul Travers, 1st Edition, 1994

> "*It is not yet clear whether they are still critical to host defense or whether they represent an evolutionary relic.*" – Janeway's Immunobiology, ed. Kenneth P. Murphy, 8th Edition, 2011

Following on from the previous meeting in Freiburg 2012 [1], the Chicago 2014 conference started off with a very special treat, namely the celebration of the 30th anniversary of the discovery of γδ T cells. The first night saw inspiring overviews given by some of the pioneers in the field who over a very short period of time made seminal contributions, from the accidental but groundbreaking cloning of an unexpected third chain of the T cell receptor (which then became the TCRγ chain) in 1984 [2,3] to the full realization by 1987 that γδ T cells in fact represent an intriguing and entirely novel lymphocyte subset [4–6]. These entertaining presentations by **Adrian Hayday** (London, UK), **Yueh-hsiu Chien** (Stanford, CA), **Ilan Bank** (Tel Aviv, Israel) and **Willi Born** (Denver, CO) included personal accounts of their research during that time and featured some of the raw data their original conclusions was based upon, which provided fascinating insight into top notch immunological research 30 years ago that is still highly relevant nowadays.

Despite outstanding research on γδ T cells ever since, these immune cells are still widely ignored and are suspiciously ill-represented in standard textbooks, as indicated by the quotes above. This negligence has traditionally been due to technical and conceptual difficulties in our understanding as to how γδ T cells are generated in the thymus, the type of target structures they recognize, and the contributions they make to homeostasis, immune surveillance, inflammation, protective immunity and autoimmunity. The Chicago 2014 conference showcased crucial advances in all these areas, as summarized in this meeting report (Fig. 1). Here we discuss some of the oral communications and a selection of poster presentations

* Corresponding author at: Cardiff Institute of Infection and Immunity, Henry Wellcome Building, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, United Kingdom. Fax: +44 29 206 87018.
E-mail address: eberlm@cf.ac.uk (M. Eberl).

http://dx.doi.org/10.1016/j.cellimm.2014.11.001
0008-8749/

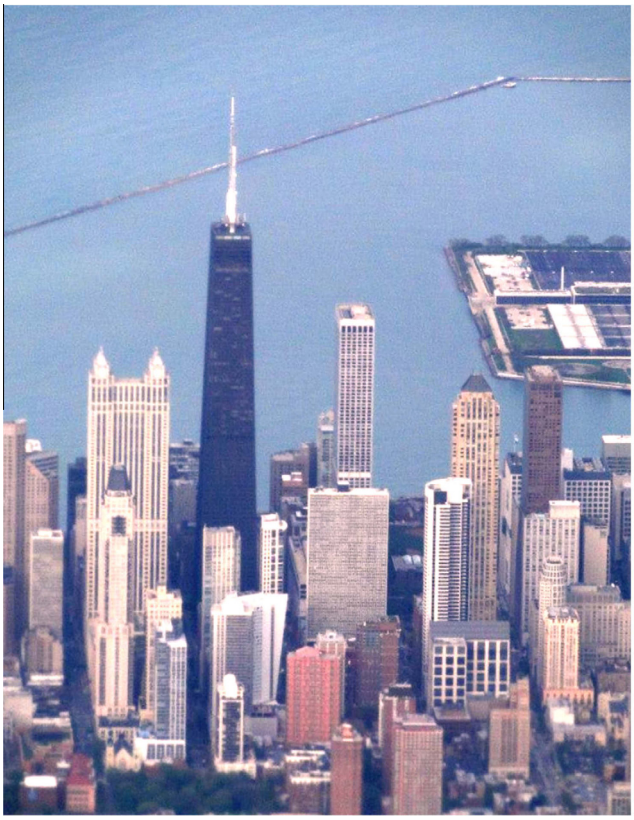

Fig. 1. View at downtown Chicago from the plane approaching O'Hare International Airport, 15th May 2014.

at the conference, and apologize in advance that many interesting contributions could not be reviewed due to space limitations. Presenting authors are highlighted in bold throughout the text.

2. γδ T cell development: thymic programming, positive selection, or peripheral expansion?

Considerable progress is being made in our understanding of the developmental pre-programming of γδ T cells, in particular of the generation of IFN-γ^+ versus IL-17^+ γδ T cells in the murine thymus [7]. **Miguel Munõz-Ruiz** from José Regueiro's laboratory (Madrid, Spain) showed that IFN-γ^+ γδ T cell numbers are significantly reduced, both in the thymus and in the periphery, in mice with diminished surface TCRγδ expression levels due to haploinsufficiency in two CD3 chains. The impaired TCR signal transduction in these mice results in a developmental blockade as indicated by the specific lack of thymic NK1.1^+ $CD27^+$ γδ T cells, suggesting that high constitutive TCRγδ expression is critical for the development of IFN-γ producing γδ T cells.

TCRγδ signaling is known to control a transcriptional network that induces Id3, which in turn inhibits the E2 transcription factor, HEBAlt. **Michelle Anderson** (Toronto, Canada) showed that HEBAlt overexpression in mice increases IL-17^+ γδ T cell numbers in the lung, whereas its loss-of-function impairs the expression of RORγt and Sox13, key factors in the IL-17^+ γδ T cell program. HEBAlt is expressed before the TCRγδ itself, and thus likely constitutes a TCR-independent transcriptional regulator of IL-17^+ γδ T cell development.

The post-transcriptional regulation of γδ T cell differentiation was addressed by **Nina Schmolka** from Bruno Silva-Santos's laboratory (Lisbon, Portugal), who showed that ablation of the microRNA network in T cell-specific Dicer knock-out mice significantly impacts on IL-17^+ but not IFN-γ^+ γδ T cell numbers. An expression analysis of $CD27^+$ versus $CD27^-$ $CCR6^+$ γδ T cells [8,9] identified a set of differentially expressed microRNAs whose individual contributions to the differentiation of IFN-γ^+ versus IL-17^+ γδ T cells are being assessed.

γδ T cell numbers in the periphery depend on homeostatic mechanisms that were addressed by two further oral communications. **Vasileios Bekiaris** (Lund, Sweden) reported a major role for the inhibitory receptor B and T lymphocyte attenuator (BTLA) in controlling $CD27^-$ IL-17^+ γδ T cells. BTLA-deficient mice accumulate $CD27^-$ IL-17^+ γδ T cells and are highly susceptible to dermatitis, which can be limited by an agonist BTLA antibody. Interestingly, BTLA expression is regulated by IL-7 and RORγt, which constitute additional key determinants of IL-17^+ γδ T cell homeostasis [10].

Yuan Zhuang (Durham, NC) generated a novel tool for γδ T cell research, a *Tcrd*-CreER mouse line that allows tamoxifen-inducible gene targeting specifically in γδ T cells. Using this strategy to delete the TCR signaling adaptor, LAT, the impact on dendritic epidermal T cells (DETC), the murine γδ T cell subset that selectively populates the skin, was assessed. Their data suggest that LAT-dependent TCR signaling is required for the wound healing activity of DETC but not for their homeostasis in the skin. Of note, it was recently shown that DETC progenitors become hyporesponsive to TCR stimulation upon "agonist selection" in the fetal thymus [11]. Future research will address the dynamics of TCR ligand expression in the skin, particularly upon stress or transformation.

3. γδ T cell recognition of target structures: antigens, ligands or activators?

Several presentations described new ligands for γδ T cells and provided compelling insights into their recognition. Earlier studies reported a unique role played by Skint1 in the selection and activation of murine DETC expressing canonical Vγ5/Vδ1 TCRs [12,13]. However, the mechanisms underlying the exquisite effects of Skint1 on this particular γδ T cell subset have remained unclear. While Skint1 has thus far been considered as an essentially intracellular molecule [14], new results presented by **Ben Willcox** (Birmingham, UK) suggest that key determinants of DETC selection are actually located on an exposed extracellular loop of Skint1, which raises the possibility for direct interactions between this molecule and the Vγ5/Vδ1 TCR.

In analogy to these observations on Skint1, another member of the extended B7 receptor family, butyrophilin 3 (BTN3, CD277), has been reported to play a crucial role in the selection of human Vγ9/Vδ2 T cells [15]. However, there are conflicting views regarding its mode of action. Data from **Gennaro de Libero** (Basel, Switzerland) suggested that BTN3 may act as a true presenting molecule for phosphoantigens such as isopentenyl pyrophosphate (IPP) and (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) [16]. In contrast, **Erin Adams** (Chicago, IL) showed that phosphoantigens instead interact with the intracellular B30.2 domain of BTN3A1, and subsequently switch BTN3 molecules from a neutral to a stimulatory conformation following inside-out signaling [17].

The latter model is supported by a new mutagenesis approach presented by **Craig Morita** (Iowa City, USA) and biochemical studies summarized by **Andrew Wiemer** (Storrs, CT) [18] that argue against phosphoantigen interaction with the extracellular part of BTN3, and indicate a primarily intracellular mode of action of phosphoantigens. If confirmed, these findings would indicate that phosphoantigens are not bona fide "antigens", i.e. are not displayed on the cell surface in the context of antigen-presenting molecules.

Further insight was provided by **Zsolt Sebestyén** from Jürgen Kuball's laboratory (Utrecht, The Netherlands) who identified Rho

GTPase prenylation as a new mechanism contributing to phosphoantigen-induced Vγ9/Vδ2 T cell activation. Since Rho GTPases are known to regulate changes in the actin cytoskeleton, they might be involved in the postulated inside-out signaling induced upon interaction between phosphoantigens and the B30.2 domain. **Gennaro de Libero** (Basel, Switzerland) described additional factors, including a particular ABC transporter, that appear to play an exquisite role in translocating phosphoantigens from inside to outside the cell. It is currently unclear whether these transporters could contribute to phosphoantigen internalization as well, to allow rapid activation of Vγ9/Vδ2 T cells by exogenous IPP or HMB-PP. Finally, analyses of CHO cell heterokaryons by **Thomas Hermann** (Würzburg, Germany) suggest a key role for undefined primate-specific molecules encoded by genes from chromosome 6 in the BTN3-mediated activation process [19].

These studies leave the issue open as to how interaction between BTN3 and phosphoantigen ultimately leads to Vγ9/Vδ2 T cell activation. Does it promote recruitment of yet undefined Vγ9/Vδ2 TCR ligands or cognate interactions between Vγ9/Vδ2 TCR by BTN3? As current studies suggest very low affinity interactions (if any) between the TCR and resting BTN3 molecules, one might posit the occurrence of conformational changes of the extracellular domain of BTN3, which in turn would enhance the affinity of Vγ9/Vδ2 TCR/BTN3 interactions. This hypothesis is appealing from an evolutionary standpoint since together with the recent developments in Skint1 biochemistry [14], it would support a conserved mode of stress sensing shared by murine DETC and human Vγ9/Vδ2 T cells.

In line with a constrained molecular context of TCRγδ antigen recognition, recent studies showed the occurrence of CD1d-reactive γδ T cells in humans and mice. At this meeting, **Derek Doherty** (Dublin, Ireland) identified human Vδ3$^+$ T cells that killed target cells through a mechanism that required CD1d but not the NKT cell agonist α-galactosylceramide [20]. **Adam Uldrich** from Dale Godfrey's laboratory (Melbourne, Australia) and **Adrienne Luoma** from Erin Adam's laboratory (Chicago, IL) described human Vδ1$^+$ γδ T cell clones displaying CD1d-restricted autoreactivity, and provided structural insights into how the γδ TCR interacts with these CD1d/glycolipid complexes [21,22]. These studies show an unusual docking mode of the γδ TCR onto CD1d complexes, and are reminiscent to the recognition of endothelial protein C receptor (EPCR) by a human γδ T cell clone reported previously [23], since EPCR shows striking structural similarities with CD1d.

Arguing against a restricted molecular context of TCRγδ recognition and lack of clear co-evolution between the TCRγδ and a limited set of related counter-receptors, studies from the teams of **Yueh-hsiu Chien** (Stanford, CA), **Julie Déchanet-Merville** (Bordeaux, France), **Willi Born** (Denver, CO) and others suggest recognition of diverse ligands, including immunoglobulin-related as well as immunoglobulin-unrelated molecules including phycoerythrin, ephrin receptor tyrosine kinase A2, annexin A2, HLA-I free heavy chain and dimerized insulin [1,24,25]. Such a view would be consistent with a more general immunoglobulin-like mode of antigen recognition by the TCRγδ, thus allowing triggering of γδ T cell responses in much broader stress-related contexts than αβ T cells. If confirmed, this would make the task of deciphering the antigen recognition repertoire of γδ T cells far more difficult than initially expected.

4. γδ T cells and their neighborhood: stimulation, inhibition and crosstalk

Like many other immune players, γδ T cells are essentially sociable cells whose performance is strongly influenced by the interactions they establish with their institutional targets (i.e., pathogens, stressed tissues, cancer cells) but also fine-tuned by the microenvironment in which these interactions occur. Two conference sessions were dedicated to the molecular and cellular mechanisms involved in the regulation of γδ T cell activation, the contribution of bystander cells, and the possibility of exploiting these interactions for therapeutic purposes.

Hongbo Shen from Zheng Chen's laboratory (Chicago, IL) showed that APC contact with Vγ9/Vδ2 T cells is required for the ability of IL-23 to induce the proliferation of HMB-PP activated Vγ9/Vδ2 T cells [26]. HMB-PP/IL-23-expanded Vγ9/Vδ2 T cells from infected macaques had multi-effector function as judged by their production of IL-17, IL-22, IL-2 and IFN-γ, with autocrine production of IFN-γ enhancing the expansion of recall-like Vγ9/Vδ2 T cells.

Mary Poupot (Toulouse, France) reported about their work to amplify Vγ9/Vδ2 T cells with highly preserved anti-tumor functions in the absence of IL-2. They focused their attention on IL-33 which amplifies both Th1 and Th2 immune responses, and concluded that IL-33 is worthy of further investigation to replace exogenous IL-2 for better safety profiles in phosphoantigen-activated Vγ9/Vδ2 T cell based clinical trials.

The functional outcome of γδ T cell responses is dictated not only by TCR engagement and co-stimulatory cytokines, but also fine-tuned by inhibitory and/or activatory co-receptors on their cell surface [27]. Inhibition or prevention of γδ T cell activation can be beneficial in selected situations such as maintenance of tolerance at the maternal–fetal interface. **Cristiana Cairo** (Baltimore, MD) showed that most adult Vγ9/Vδ2 T cells express CD70 after stimulation whereas CD70 expression by neonatal Vγ9/Vδ2 T cells is tightly regulated, thereby possibly contributing to limiting inflammatory responses.

Inhibition of inflammatory γδ T cell responses might be beneficial in cord blood but can be deleterious in cancer patients or other clinical settings. **Daniel Olive** (Marseille, France) provided evidence that BTLA on the cell surface of Vγ9/Vδ2 T cells impairs their ability to respond to TCR-independent or TCR-dependent stimulation via interaction with herpes virus entry mediator (HVEM) expressed by lymphoma cells. Interference with the BLTA/HVEM axis could thus be a possible strategy to recover anti-tumor immune functions of Vγ9/Vδ2 T cells in lymphoma patients.

Other reports significantly enriched the co-signaling landscape by providing new data on regulatory pathways operative in γδ T cells. **Janice Telfer** and **Cynthia Baldwin** (Amherst, MA) addressed the function of WC1 proteins on bovine γδ T cells [28]. These proteins are members of the scavenger receptor cysteine-rich (SRCR) superfamily that have the potential to act as both co-stimulatory receptors and pattern recognition receptors for ligands expressed by spirochetes and mycobacteria. In the murine system, expression of Skint1 by thymic epithelial cells and keratinocytes is essential for the selective development of DETCs [29]. **Anett Jandke** from Adrian Hayday's laboratory (London, UK) provided evidence suggesting that DETC development and peripheral expansion in the skin is regulated by both Skint1 and the related molecule Skint2 as epithelial determinants of DETCs at steady state and upon stress.

Besides through the action of inhibitory and costimulatory co-receptors, homeostasis, activation and functional differentiation of γδ T cells are regulated by the tight interplay with bystander cells in the local microenvironment. Intraepithelial lymphocytes (IEL), which include both αβ and γδ T cells, represent an important barrier in the prevention of infection against enteric pathogens. **Karen Edelblum** (Chicago, IL) reported that γδ IEL migration and interaction with the intestinal epithelium via occludin and CD103 are essential to the maintenance of mucosal homeostasis and immediate host response to intestinal pathogens. Other important IEL regulators are proteins encoded by butyrophilin-like proteins [30]. **Cristina Lebrero-Fernández** from Anna Bas Fors-

berg's laboratory (Göteborg, Sweden) showed that Btnl6 and Btnl4 form heteromeric protein complexes with Btnl1, and that these complexes can induce proliferation of IELs in the presence of IL-2 and in the absence of TCR engagement, indicating that Btnl proteins contribute to the functional regulation of T cell mediated gut immunity.

Keith Thompson (Aberdeen, UK) confirmed the capacity of Vγ9/Vδ2 T cells to interact with cells outside the immune system by showing unexpected interactions between osteoclasts and γδ T cells leading to enhanced activation of γδ T cells on one hand and suppression of osteoclast differentiation and function on the other hand, suggesting an involvement of Vγ9/Vδ2 T cells in the inhibition of bone resorption upon administration of aminobisphosphonates [31].

The complexity of interactions between γδ T cells and other immune and non-immune cells in normal, inflamed, or cancer tissues can represent a major constraint to develop strategies to specifically activate and locally exploit distinct subsets of γδ T cells. To this end, **Anna Capsomidis** from John Anderson's laboratory (London, UK) reported a comprehensive analysis of the expression of costimulatory receptor-ligand pairs on Vδ1$^+$, Vδ2$^+$, and non-Vδ1/non-Vδ2 T cells after 28 days of incubation with K562-derived artificial antigen presenting cells (aAPCs) [32]. Their results show that this assay allows the expansion of different γδ T cell subsets in sufficient amounts for subsequent *in vitro* and *in vivo* functional analyses.

The potential of γδ T cells to orchestrate immune responses was strengthened by **Andreea Petrasca** from Derek Doherty's laboratory (Dublin, Ireland) who showed that Vγ9/Vδ2 T cells can induce maturation of both B cells and dendritic cells (DC) into antigen-presenting cells (APCs). Interestingly, Vγ9/Vδ2 T cells prime B cells to stimulate Th2 responses, whereas they prime DC to stimulate Th1 responses. **Matt Morgan** from Matthias Eberl's laboratory (Cardiff, UK) showed that Vγ9/Vδ2 T cells and MAIT cells respond to human neutrophils after phagocytosis of microbial pathogens, and in turn mediate the differentiation of bystander neutrophils into APCs for both CD4$^+$ and CD8$^+$ T cells [33]. Taken together these findings support the existence of a peripheral immune surveillance network comprised of unconventional T cells and neighboring immune and non-immune cells [34], and identify a unique and decisive role for human γδ T cells in shaping the transition of the innate to the adaptive phase of immune responses.

5. γδ T cells in infection: regulation, exacerbation, or protection?

The partaking of γδ T cells in the riposte to pathogens is widely recognized in many different infectious scenarios in mouse models and in human pathologies. There is undeniable evidence for the expansion of particular γδ T cell subsets in the blood or in infected tissues. However, dissecting whether these cells have a genuine anti-infectious function and are involved in protective immunity, exacerbate the inflammatory response and hence the pathogenicity, or act as general immune regulators is a challenging task, especially *in vivo*.

Over the last years, advances in our understanding of the anti-infectious function of γδ T cells have been made by the team of Zheng Chen (Chicago, IL) (Fig. 2) through studies in a macaque model of tuberculosis [35]. In this meeting, **Arwa Qaqish** from this laboratory exposed the capacity of adoptively transferred autologous Vγ9/Vδ2 T cells to control an ongoing *Mycobacterium tuberculosis* infection and to limit disease lesions in lungs, where Vγ9/Vδ2 T cells were detected early after infection. In an alternative model to study the effect of Vγ9/Vδ2 T cells *in vivo*, **Suzanne Tomchuck** from Mari Dallas's team (Memphis, TN) showed a beneficial role

Fig. 2. Conference organizer Prof. Zheng W. Chen from the University of Illinois College of Medicine in Chicago during his welcome speech, 16th May 2014.

of adoptively transferred human cord blood Vγ9/Vδ2 T cells activated by zoledronate on the survival of NSG mice infected with *Streptococcus pneumoniae*.

Two further studies demonstrated a protective role of murine γδ T cells against murine cytomegalovirus (MCMV) upon priming with MCMV *in vivo* and transfer into immunodeficient mice, in extension of the previously described response of human γδ T cells to CMV. In this model, **Camille Khairallah** from Julie Déchanet-Merville's laboratory (Bordeaux, France) showed the response of spleen, liver and lung Vγ1$^+$ and Vγ4$^+$ T cells acquiring a memory phenotype but strikingly low expression of IL-17, IFN-γ and cytotoxic potential. **Sabrina Sell** from Thomas Winkler's and Michael Mach's laboratories (Erlangen, Germany) demonstrated a better control of viral spread in wild type mice when compared to TCRδ$^{-/-}$ mice, uncovering a non-redundant function of γδ T cells against this virus. The MCMV model might be particularly valuable to analyze the potential cooperation of γδ T cells with other cells involved in viral control such as NK cells.

In vitro investigations by different teams aimed to decipher the mechanisms of pathogen recognition by γδ T cells and their mode of action. **Christina Maher** from Derek Doherty's laboratory (Dublin, Ireland) reported an IL-23 dependent expansion of IL-17-producing Vδ1$^+$ T cells cultured in contact with *Candida albicans* exposed DC. In an elegant model of latent HIV infection, **Haishan Li** from David Pauza's laboratory (Baltimore, MD) demonstrated the killing capacity of Vγ9/Vδ2 T cells against CD4$^+$ T cells reactivating a latent virus, opening up perspectives for the eradication of the latent HIV reservoir. *M. tuberculosis* produces HMB-PP, the phosphoantigen with the strongest agonistic activity on Vγ9/Vδ2 T cells. However, by analysing the differential responses of Vγ9/Vδ2 T cells to phosphoantigens and to bacillus Calmette Guérin (BCG), **Daniel Hoft** (St. Louis, CO) provided evidence for the exis-

tence of a novel agonist for Vγ9/Vδ2 T cells with anti-bacterial activity, contained within methyl-glucose lipopolysaccharides extracted from BCG.

The ultimate goal of all these studies on infectious models should be to elaborate novel immunotherapies and vaccines based on γδ T cell activation [36]. An interesting option in that direction was proposed by **Hong Wang** from Craig Morita's laboratory (Iowa City, IA), who engineered an attenuated strain of *Salmonella enterica* producing high levels of HMB-PP which elicited a prolonged expansion of Vγ9/Vδ2 T cells, *ex vivo* and in rhesus monkeys [37]. Given their rapid expansion and immediate effector functions during the early response to microorganisms, the evaluation of γδ T cells (and other unconventional T cells such as MAIT cells) in infected patients might also represent reliable diagnostic or prognostic biomarkers [38]. In this context, **Anna Rita Liuzzi** from Matthias Eberl's laboratory (Cardiff, UK) demonstrated the diagnostic value of local Vγ9/Vδ2 T cells as specific predictors of acute Gram-negative infections and poor clinical outcome in peritoneal dialysis patients [39].

6. γδ T cells in the tissue: immune surveillance, wound healing, and autoimmunity

γδ T cells play key roles in the regulation of inflammation through cytokine production and cell–cell crosstalk. While inflammation is necessary for the promotion of tissue repair and protection from infection, chronic inflammatory responses contribute to a variety of conditions including non-healing wounds, cancer and autoimmunity. Thus, γδ T cells can tip the balance from a healthy inflammatory response to a destructive disease and as such represent important therapeutic targets.

Ever since γδ T cells were found to promote tissue repair in a murine model of wound repair over a decade ago [40], there has been controversy about whether or not a similar population exists in human skin. $V\delta1^+$ T cells in the human epidermis were previously shown to produce IGF-1 and become activated in wounded skin [41]. Now, **Richard Woolf** from Adrian Hayday's laboratory (London, UK) demonstrated that human $V\delta1^+$ T cells in the skin express and are activated via the NKG2D receptor. In fact these cells are the only T cell population in the skin with this innate-like activation phenotype.

Murine DETC recognize an as yet unidentified antigen that requires JAML costimulation for wound healing functions [42]. **Kevin Ramirez** from Wendy Havran's laboratory (La Jolla, CA) identified another player in the activation of this γδ T cell population. EPCR has been shown to play roles in human γδ T cell responses to CMV [23]. They now demonstrate that EPCR is upregulated on murine keratinocytes at wound sites, and that EPCR is required for normal wound repair and epidermal γδ T cell function.

To better understand signaling involved in DETC wound healing functions, **Shelley Dutt** from Julie Jameson's laboratory (San Marcos, CA) investigated the role of the signaling molecule mammalian target of rapamycin (mTOR). Murine models of wound repair in the Jameson laboratory have shown that mTOR is required for epidermal γδ T cell function in wound closure. They are now turning their attention to human studies to determine whether there is a concentration of mTOR inhibition that impairs T cell-mediated allograft rejection but allows for proper γδ T cell wound healing functions.

Tissue repair and inflammation are balanced by the potential damage that dysregulated T cell responses can cause. Several laboratories attempt to delineate the roles played by γδ T cells in modulating tissue remodeling and related disease. **Shubhada Chiplunkar** (Mumbai, India) reported that patients with gallbladder cancer display alterations in the dynamics of Tγδ17/Treg ratios. Indeed patients with an increased frequency of Tγδ17 cells exhibit poor survival, exemplifying the need to define ways to regulate the polarization of γδ T cell responses in disease. **Christopher Tyler** from Matthias Eberl's laboratory (Cardiff, UK) demonstrated that γδ T cells are able to regulate the inflammatory environment via their ability to present antigens and promote IFN-γ and IL-22 production by $CD4^+$ T cells, suggesting that the cellular microenvironment dictates the ability of γδ T cells to modulate naïve and memory T cell responses.

Xiaoyang Wang (Göteborg, Sweden) addressed the "dark side" of γδ T cell responses in a murine model of brain injury. While γδ T cells infiltrate the brain of wildtype mice within 6 h of hypoxia-ischemia induction, mice lacking γδ T cells exhibit reduced gray-matter volume loss suggesting that they contribute to neonatal brain injury. Similarly, studies by **Victoria Marcu-Malina** from Ilan Bank's laboratory (Tel Aviv, Israel) identified an immunopathogenic role for γδ T cells in systemic sclerosis upon zoledronate activation. In addition, a role for IL-17 producing γδ T cells in ankylosing spondylitis is being investigated by **Annika Reinhardt** from Immo Prinz's laboratory (Hannover, Germany). Entheseal $CCR6^+$ $IL\text{-}17^+$ γδ T cells represent a newly defined tissue-resident population with the ability to regulate inflammatory responses. Taken together, this session underscored the diverse functions of γδ T cells and their promising potential as therapeutic targets.

7. γδ T cells in cancer: immunotherapy versus tumor progression

Almost since their discovery, γδ T cells have long been known to mediate anticancer cytotoxicity. Here, **Daniel Olive** (Marseille, France) reported that human Vγ9/Vδ2 T cells activated by agonist anti-BTN3A antibodies show a strong activity versus acute myeloid leukemia cells. **Gauri Mirji** from Shubhada Chiplunkar's laboratory (Mumbai, India) showed that both human $V\delta1^+$ and $V\delta2^+$ T cells are cytolytic for T cell acute lymphoblastic leukemia. **Mohanad Nada** from Craig Morita's laboratory (Iowa City, IA) and **Ulrich Jarry** from Emmanuel Scotet's laboratory (Nantes, France) confirmed that human γδ T cells activated by a combination of zoledronate plus IL-15, or local administration of zoledronate plus autologous Vγ9/Vδ2 T cells, represent straightforward tools for γδ T cell-based cell therapies of brain and ovarian tumors, and beyond. **Hung-Chang Chen** from Matthias Eberl's laboratory (Cardiff, UK) identified a powerful synergism between Vγ9/Vδ2 T cells and antigen-specific $CD8^+$ T cells in the eradication of human tumor cells including breast cancer stem cells, suggesting the potential of novel two-pronged immunotherapy strategies that combine non-MHC restricted and MHC restricted mechanisms.

Helping γδ T cells to bind and kill cancer target cells by a combination of activating phosphoantigens and anticancer therapeutic monoclonal antibodies had been investigated for non-Hodgkin lymphoma and breast carcinoma, but the combination regimen only starts being assessed in clinical trials [43]. In this regard, an interesting new option was proposed by **Daniela Wesch** (Kiel, Germany), who reported that a novel reagent called $[(Her2)_2 \times V\gamma9]$ tribody induces an important bioactivity against pancreatic ductal adenocarcinoma (PDAC), for which little if any treatment is available to patients [44]. A distinct yet related approach was presented by **Jonathan Fisher** from John Anderson's laboratory (London, UK), namely that human $V\delta2^+$ T cells engineered to express a chimeric antigen receptor targeting the ganglioside GD2 show potent effector functions against neuroblastoma and Ewing sarcoma.

In real life however, most tumors develop immune escape strategies that are already in action upon diagnosis. Hence, γδ T cells have to face these successive pitfalls to successfully play their good role. **Daniel Olive** (Marseille, France) illustrated this by showing

that the induced expression of BTLA is one such escape pathway evolved by non-Hodgkin lymphomas. Likewise, **Massimo Massaia** (Torino, Italy) reported that PD-1 plays a similar role and therefore that its blockade by targeting either PD-1$^+$ γδ cells or PD-L1$^+$ myeloid-derived suppressor cells can restore human γδ T cell responses against multiple myeloma. Furthermore, **Daniel Gonnermann** from Daniela Wesch's laboratory (Kiel, Germany) showed that prostaglandin E_2, a previously described inhibitory factor for human γδ T cells [45], contributes to dampen their cytotoxicity for PDAC.

Finally, a pejorative role for γδ T cells is also part of the whole picture, as under some yet poorly characterized circumstances these cells could promote not only immunity but also progression of cancer [46,47], such as human papillomavirus-driven cervix carcinoma, as presented by **Nathalie Jacobs** (Liège, Belgium). Intriguingly, **Margarida Rei** from Bruno Silva-Santos's (Lisbon, Portugal) and Daniel Pennington's laboratories (London, UK) showed that IL-17$^+$ γδ T cells can be deleterious in emergence of murine ovarian cancer, possibly via secretion of pro-angiogenic and pro-inflammatory factors by small peritoneal macrophages [48], in line with **Shubhada Chiplunkar's** (Mumbai, India) findings in gallbladder cancer patients.

8. Concluding remarks

Being held 30 years after the original discovery of this enigmatic lymphocyte subset, the 6th γδ T cell conference in Chicago 2014 provided an inspiring overview of current research in the field, with significant advances being reported as to their contribution to immune responses in a wide range of scenarios. Particularly noteworthy are the exciting advances and encouraging efforts to apply this newly gained knowledge in the clinic in patients with infections, autoimmune disorders and cancer. Although there is still much to be learned about γδ T cells and the regulatory and effector roles they exert in the immune system, they have finally come of age and emancipated themselves as third and independent lymphocyte lineage whose manifold functions and unique reactivities elegantly complement and extend those of conventional αβ T cells and B cells and are critical to host defense – an evolutionary relic no more.

The 7th γδ T cell conference will be organized by **Adrian Hayday** and is scheduled for 2016 in London (UK).

Acknowledgments

We thank all researchers cited in this report for their input and sincerely apologize to those who could not be covered due to length restrictions. We are especially grateful to Linda Barrows for website design and help with organizing the conference, and to Arwa Qaqish, Crystal Chen, Dan Huang and Ling Shen for general assistance. This conference was generously sponsored by NIH grant R13 AI109968 and various commercial sponsors. M.M. acknowledges travel support from the Associazione Italiana per la Ricerca sul Cancro. M.E. acknowledges travel awards from the British Society for Immunology – UK and Cardiff University's William Morgan Thomas Fund – UK, and the members of his laboratory for critical comments on this manuscript.

References

[1] B. Silva-Santos, W.W. Schamel, P. Fisch, M. Eberl, γδ T cell conference 2012: close encounters for the fifth time, Eur. J. Immunol. 42 (2012) (2012) 3101–3105.
[2] H. Saito, D.M. Kranz, Y. Takagaki, A.C. Hayday, H.N. Eisen, S. Tonegawa, Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences, Nature 309 (1984) 757–762.
[3] A.C. Hayday, H. Saito, S.D. Gillies, D.M. Kranz, G. Tanigawa, H.N. Eisen, et al., Structure, organization, and somatic rearrangement of T cell gamma genes, Cell 40 (1985) 259–269.
[4] I. Bank, R.A. DePinho, M.B. Brenner, J. Cassimeris, F.W. Alt, L. Chess, A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes, Nature 322 (1986) 179–181.
[5] W. Born, C. Miles, J. White, R. O'Brien, J.H. Freed, P. Marrack, et al., Peptide sequences of T-cell receptor delta and gamma chains are identical to predicted X and gamma proteins, Nature 330 (1987) 572–574.
[6] E.Y. Loh, L.L. Lanier, C.W. Turck, D.R. Littman, M.M. Davis, Y.H. Chien, et al., Identification and sequence of a fourth human T cell antigen receptor chain, Nature 330 (1987) 569–572.
[7] I. Prinz, B. Silva-Santos, D.J. Pennington, Functional development of γδ T cells, Eur. J. Immunol. 43 (2013) 1988–1994.
[8] J.C. Ribot, A. deBarros, D.J. Pang, J.F. Neves, V. Peperzak, S.J. Roberts, et al., CD27 is a thymic determinant of the balance between interferon-γ- and interleukin 17-producing γδ T cell subsets, Nat. Immunol. 10 (2009) 427–436.
[9] J.D. Haas, F.H. González, S. Schmitz, V. Chennupati, L. Föhse, E. Kremmer, et al., CCR6 and NK1.1 distinguish between IL-17A and IFN-γ-producing γδ effector T cells, Eur. J. Immunol. 39 (2009) 3488–3497.
[10] V. Bekiaris, J.R. Sedý, M.G. Macauley, A. Rhode-Kurnow, C.F. Ware, The inhibitory receptor BTLA controls γδ T cell homeostasis and inflammatory responses, Immunity 39 (2013) 1082–1094.
[11] M. Wencker, G. Turchinovich, R. Di Marco Barros, L. Deban, A. Jandke, et al., Innate-like T cells straddle innate and adaptive immunity by altering antigen-receptor responsiveness, Nat. Immunol. 15 (2014) 80–87.
[12] J.M. Lewis, M. Girardi, S.J. Roberts, S.D. Barbee, A.C. Hayday, R.E. Tigelaar, Selection of the cutaneous intraepithelial γδ$^+$ T cell repertoire by a thymic stromal determinant, Nat. Immunol. 7 (2006) 843–850.
[13] L.M. Boyden, J.M. Lewis, S.D. Barbee, A. Bas, M. Girardi, A.C. Hayday, et al., Skint1, the prototype of a newly identified immunoglobulin superfamily gene cluster, positively selects epidermal γδ T cells, Nat. Genet. 40 (2008) 656–662.
[14] S.D. Barbee, M.J. Woodward, G. Turchinovich, J.J. Mention, J.M. Lewis, L.M. Boyden, et al., Skint-1 is a highly specific, unique selecting component for epidermal T cells, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 3330–3335.
[15] C. Harly, Y. Guillaume, S. Nedellec, C.M. Peigné, H. Mönkkönen, J. Mönkkönen, et al., Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T cell subset, Blood 120 (2012) 2269–2279.
[16] S. Vavassori, A. Kumar, G.S. Wan, G.S. Ramanjaneyulu, M. Cavallari, S. El Daker, et al., Butyrophilin 3A1 binds phosphorylated antigens and stimulates human γδ T cells, Nat. Immunol. 14 (2013) 908–916.
[17] A. Sandstrom, C.M. Peigné, A. Léger, J.E. Crooks, F. Konczak, M.C. Gesnel, et al., The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vγ9/Vδ2 T cells, Immunity 40 (2014) 490–500.
[18] C.H. Hsiao, X. Lin, R.J. Barney, R.R. Shippy, J. Li, O. Vinogradova, et al., Synthesis of a phosphoantigen prodrug that potently activates Vγ9Vδ2 T-lymphocytes, Chem. Biol. (2014), http://dx.doi.org/10.1016/j.chembiol.2014.06.006.
[19] F. Riaño, M.M. Karunakaran, L. Starick, J. Li, C.J. Scholz, V. Kunzmann, et al., Vγ9Vδ2 TCR-activation by phosphorylated antigens requires butyrophilin 3 A1 (BTN3A1) and additional genes on human chromosome 6, Eur. J. Immunol. 21 (2014) 945–954.
[20] B.A. Mangan, M.R. Dunne, V.P. O'Reilly, P.J. Dunne, M.A. Exley, D. O'Shea, et al., CD1d restriction and Th1/Th2/Th17 cytokine secretion by human Vδ3 T cells, J. Immunol. 191 (2013) 30–34.
[21] A.P. Uldrich, J. Le Nours, D.G. Pellicci, N.A. Gherardin, K.G. McPherson, R.T. Lim, et al., CD1d-lipid antigen recognition by the γδ TCR, Nat. Immunol. 14 (2013) 1137–1145.
[22] A.M. Luoma, C.D. Castro, T. Mayassi, L.A. Bembinster, L. Bai, D. Picard, et al., Crystal structure of Vδ1 T cell receptor in complex with CD1d-sulfatide shows MHC-like recognition of a self-lipid by human γδ T cells, Immunity 39 (2013) 1032–1042.
[23] C.R. Willcox, V. Pitard, S. Netzer, L. Couzi, M. Salim, T. Silberzahn, et al., Cytomegalovirus and tumor stress surveillance by binding of a human γδ T cell antigen receptor to endothelial protein C receptor, Nat. Immunol. 13 (2012) 872–879.
[24] X. Zeng, Y.L. Wei, J. Huang, E.W. Newell, H. Yu, B.A. Kidd, et al., Γδ T cells recognize a microbial encoded B cell antigen to initiate a rapid antigen-specific interleukin-17 response, Immunity 37 (2012) 524–534.
[25] M.K. Aydintug, L. Zhang, C. Wang, D. Liang, J.M. Wands, A.W. Michels, et al., Γδ T cells recognize the insulin B:9–23 peptide antigen when it is dimerized through thiol oxidation, Mol. Immunol. 60 (2014) 116–128.
[26] H. Shen, Y. Wang, C.Y. Chen, J. Frencher, D. Huang, E. Yang, et al., Th17-related cytokines contribute to recall-like expansion/effector function of HMBPP-specific Vγ2Vδ2 T cells after *M. tuberculosis* infection or vaccination, Eur. J. Immunol. (2014), http://dx.doi.org/10.1002/eji.201444635.
[27] J.C. Ribot, A. deBarros, B. Silva-Santos, Searching for "signal 2": costimulation requirements of γδ T cells, Cell. Mol. Life Sci. 68 (2011) 2345–2355.
[28] C. Chen, H. Hsu, E. Hudgens, J.C. Telfer, C.L. Baldwin, Signal transduction by different forms of the γδ T cell-specific pattern recognition receptor WC1, J. Immunol. 193 (2014) 379–390.
[29] S.D. Barbee, M.J. Woodward, G. Turchinovich, J.J. Mention, J.M. Lewis, L.M. Boyden, et al., Skint-1 is a highly specific, unique selecting component for epidermal T cells, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 3330–3335.
[30] L. Abeler-Dörner, M. Swamy, G. Williams, A.C. Hayday, A. Bas, Butyrophilins: an emerging family of immune regulators, Trends Immunol. 33 (2012) 34–41.

[31] A. Pappalardo, K. Thompson, Activated γδ T cells inhibit osteoclast differentiation and resorptive activity in vitro, Clin. Exp. Immunol. 174 (2013) 281–291.
[32] J. Fisher, M. Yan, J. Heuijerjans, L. Carter, A. Abolhassani, J. Frosch, et al., Neuroblastoma killing properties of V-delta 2 and V-delta2 negative gamma delta T cells following expansion by artificial antigen presenting cells, Clin. Cancer Res. (2014) in press; http://dx.doi.org/10.1158/1078-0432.CCR-13-3464.
[33] M.S. Davey, M.P. Morgan, A.R. Liuzzi, C.J. Tyler, M.W.A. Khan, T. Szakmany, et al., Microbe-specific unconventional T cells induce human neutrophil differentiation into antigen cross-presenting cells, J. Immunol. 193 (2014) 3704–3716.
[34] A.C. Hayday, γδ T cells and the lymphoid stress-surveillance response, Immunity 31 (2009) 184–196.
[35] C.Y. Chen, S. Yao, D. Huang, H. Wei, H. Sicard, G. Zeng, et al., Phosphoantigen/IL2 expansion and differentiation of Vγ2Vδ2 T cells increase resistance to *tuberculosis* in nonhuman primates, PLoS Pathog. 9 (2013) e1003501.
[36] Y.H. Chien, C. Meyer, M. Bonneville, γδ T cells: first line of defense and beyond, Annu. Rev. Immunol. 32 (2014) 121–155.
[37] G. Workalemahu, H. Wang, K.J. Puan, M.H. Nada, T. Kuzuyama, B.D. Jones, et al., Metabolic engineering of Salmonella vaccine bacteria to boost human Vγ2Vδ2 T cell immunity, J. Immunol. 193 (2014) 708–721.
[38] M. Eberl, I.M. Friberg, A.R. Liuzzi, M.P. Morgan, N. Topley, Pathogen-specific immune fingerprints during acute infection: the diagnostic potential of human γδ T-cells, Front. Immunol. 5 (2014) 572.
[39] C.Y. Lin, G.W. Roberts, A. Kift-Morgan, K.L. Donovan, N. Topley, M. Eberl, Pathogen-specific local immune fingerprints diagnose bacterial infection in peritoneal dialysis patients, J. Am. Soc. Nephrol. 24 (2013) 2002–2009.
[40] J. Jameson, K. Ugarte, N. Chen, P. Yachi, E. Fuchs, R. Boismenu, et al., A role for skin γδ T cells in wound repair, Science 296 (2002) 747–749.
[41] A. Toulon, L. Breton, K.R. Taylor, M. Tenenhaus, D. Bhavsar, C. Lanigan, et al., A role for human skin-resident T cells in wound healing, J. Exp. Med. 206 (2009) 743–750.
[42] D.A. Witherden, P. Verdino, S.E. Rieder, O. Garijo, R.E. Mills, L. Teyton, et al., The junctional adhesion molecule JAML is a costimulatory receptor for epithelial γδ T cell activation, Science 329 (2010) 1205–1210.
[43] J. Gertner-Dardenne, C. Bonnafous, C. Bezombes, A.H. Capietto, V. Scaglione, S. Ingoure, et al., Bromohydrin pyrophosphate enhances antibody-dependent cell-mediated cytotoxicity induced by therapeutic antibodies, Blood 113 (2009) 4875–4884.
[44] H.H. Oberg, M. Peipp, C. Kellner, S. Sebens, S. Krause, D. Petrick, et al., Novel bispecific antibodies increase γδ T-cell cytotoxicity against pancreatic cancer cells, Cancer Res. 74 (2014) 1349–1360.
[45] L. Martinet, S. Fleury-Cappellesso, M. Gadelorge, G. Dietrich, P. Bourin, J.J. Fournié, et al., A regulatory cross-talk between Vγ9Vδ2 T lymphocytes and mesenchymal stem cells, Eur. J. Immunol. 39 (2009) 752–762.
[46] S. Ma, Q. Cheng, Y. Cai, H. Gong, Y. Wu, X. Yu, et al., IL-17A produced by γδ T cells promotes tumor growth in hepatocellular carcinoma, Cancer Res. 74 (2014) 1969–1982.
[47] P. Wu, D. Wu, C. Ni, J. Ye, W. Chen, G. Hu, et al., ΓδT17 cells promote the accumulation and expansion of myeloid-derived suppressor cells in human colorectal cancer, Immunity 40 (2014) 785–800.
[48] M. Rei, N. Gonçalves-Sousa, T. Lança, R.G. Thompson, S. Mensurado, F.R. Balkwill, et al., Murine $CD27^{(-)}$ $V\gamma6^{(+)}$ γδ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) E3562–E3570.